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Effects of Sodium 2-Mercaptoethanesulfonate (Mesna) as a Post-treatment in Mice Challenged with Phosgene

Jeffry S. Forster
Steven I. Baskin
Theodore S. Moran
Mathew P. Cascio
Traci L. Graham
Alfred M. Sciuto

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U.S. Army Medical Research
Institute of Chemical Defense
Aberdeen Proving Ground, MD 21010-5400

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Jeffry S. Forster, Steven I. Baskin , Theodore S. Moran, Mathew P. Cascio, Traci L. Graham, Alfred M. Sciuto

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US Army Medical Research Institute of Chemical Defense
ATTN: MCMR-UV-PN
3100 Ricketts Point Road
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ATTN: MCMR-UV-RC
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14. ABSTRACT The effects of 2-mercaptoethanesulfonate (mesna) as a post-treatment against exposure to a lethal dose of phosgene (CG) were investigated. Mesna appears to be a potential candidate against phosgene-induced injury because it may increase sulfhydryl concentrations, which may reduce cellular injury. Mice were exposed to phosgene, 32 mg/m^3, or air for 20 minutes. After a 5-minute washout with air, mice were injected interperitoneally (ip) with 0, 334, 834 or 1,667 mg/kg of mesna or phosphate buffered saline (PBS) at 20 minutes, 2, 5, 8 and 11 hours. Survival was assessed every 2 hours for 12 hours and at 24 hours. Lungs were removed and analyzed for wet weight, dry weight, wet/dry ratio, protein, and non-protein sulfhydryls (NPSH). These parameters were measured up to 12 hours after exposure. Air-exposed mice (treated equivalently) were also sacrificed. Survival rates were increased in the 834 mg/kg mesna + CG group through eight hours over the PBS + CG group. Only at the 8-hour time point did the 1,667 mg/kg mesna + CG dose show an increase in survival rates compared with PBS + CG control. Wet weight, dry weight, and wet/dry ratio were not statistically different from CG + PBS controls for all groups. Non-protein sulfhydryls did, however, show an increase at the 1,667 mg/kg dose at the 12-hour time point. These data suggest that in this paradigm, thiols only play a minor role in phosgene toxicity. In conclusion, while mesna shows some degree of protection against CG, the large doses required to afford this protection may offer only a narrow dose range window of therapeutic opportunity for the treatment of phosgene.

15. SUBJECT TERMS
Mesna, 2-Mercaptoethanesulfonate, Phosgene, Lung Edema

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Introduction

2-Mercaptoethanesulfonate (mesna) (Mesnex®) (Figure 1) is used clinically, most notably as an adjunct in cancer chemotherapy.[1,2] Mesna, by virtue of its thiol group, appears to bind to and detoxify various metabolites of chemotherapeutic drugs, rendering them harmless and allowing the body to excrete them without damage.[3] Also known as Uromitexan®, mesna appears to reduce the viscosity of mucus, aiding mucociliary clearance[1] and may have antioxidant properties.[4,5] Mesna may also increase levels of glutathione (GSH) (Figure 2), a water-soluble thiol. It is this thiol-containing tripeptide and its ability to form conjugates with toxic chemicals that make GSH important in protecting cells against reactive O_2 and free radicals.[6] GSH also protects proteins by preventing oxidation of SH groups and by reducing disulfide bonds caused by oxidative stress.[7]

Phosgene (CG) is a toxic gas that was used extensively in World War I resulting in vast numbers of injuries and deaths. Today it is used in the synthesis of insecticides, plastics, pharmaceutical drugs and dyes.[8] The National Institute for Occupational Safety and Health (NIOSH) reports that over one million tons of phosgene are produced annually in the United States and that thousands of workers may be involved with its production and usage.[9] CG is usually completely consumed in most of these processes; however, an accident or spill could lead to serious medical and occupational problems for industrial workers or firefighters. Inhalation of CG can cause non-cardiogenic pulmonary edema and possibly death, within 6-24 hours after exposure.[10] In this study we evaluated the therapeutic efficacy of a post-exposure administration of mesna against a toxic concentration of CG. We measured survival ratios every two hours for 12 hours and at 24 hours. Non-protein sulfhydryls were measured also as an indication of protective thiol groups.

Methods

Male CD-1 (Charles River, Wilmington, MA) mice weighing 25-30g were exposed whole-body to 32 mg/m^3 phosgene for 20 min (640 mg·min/m^3). All elements of exposure were performed in an approved laboratory fume hood. Ten percent phosgene:balance N_2 (Matheson Gas Products, Baltimore, MD) was metered through a Tylan mass flow controller (Tylan Corp., Torrance, CA) at a rate of 20 L/min. This was mixed with room air and then passed through an infrared spectrometer (Miran 1A, Foxboro Co., Sharon, MA). The Miran 1A was equipped with a real-time analog output. Concentration versus time graphs were developed, and the input concentration was calculated. The exposure occurred in a Plexiglas cylinder (25 cm in height x 28 cm in diameter) with a total volume of 15.8 L. The chamber was divided into 4 quadrants with 10 mice per quadrant. Exposure to phosgene was for 20 minutes followed by a 5-minute chamber air washout. Outflowing gas from the chamber was passed through a second Miran 1A unit to determine the concentration of phosgene exiting the chamber. Following the exposure, the mice were injected (IP) with appropriate mesna (334, 834 or 1,667 mg/kg) or PBS treatments at 20 minutes, 2, 5, 8 and 11 hours after being removed from the chamber, to maintain a constant level of mesna. In all, each mouse received 5 injections of the appropriate concentration of mesna. Mesna was prepared fresh in PBS

the day of the experiment and each injection consisted of 0.5 ml of solution. Mice were exposed in two groups of 40, one group receiving phosgene and the other group receiving air. This regimen was performed on three separate occasions. Number of mice and how they were grouped are in Table 1 below.

Table 1

Mesna (mg/kg)	AIR	CG
0 (PBS)	42	42
334	13	13
834	39	39
1667	26	26

Mice were observed for 12 hours from the end of exposure. Mice that expired within 12 hours were weighed immediately and necropsied for their lung tissue. The left lung was weighed and placed on a tared planchete for wet/dry weight ratios. Lungs for the wet/dry ratio were placed in an oven at 100°C and reweighed eight days later for dry weight. The entire right lung was quickly frozen in liquid nitrogen and stored at -80°C for future biochemical measurements. All lung weight gravimetric data are from mice that expired at 12 hours after the start of exposure. There are no gravimetric parameters or NPSH data from lung tissue of mice that died after 12 hours because these tissues were not fresh.

Since the mass of individual mouse lungs was small, we pooled lung tissue within the same drug treatment groups. Three to four mouse lungs were pooled for each treatment dose per assay since this enabled us to adequately perform assays in duplicate. All assays were performed on pooled mouse lung tissue, which was ground to a fine powder under liquid nitrogen.

As an estimate of protective thiol concentrations, nonprotein bound sulfhydryls were measured using the method outlined by Sedlak and Lindsay.[11] Briefly, 75 mg of pooled frozen lung tissue was homogenized in 0.02 M EDTA disodium salt and mixed with distilled water and 15% TCA. Next, these homogenates were mixed with 0.4 M tris buffer and 0.2 M EDTA disodium salt, pH 8.9, and 100 µl of dithio-bis-[2-nitrobenzoic acid] (DTNB) and slowly shaken at room temperature for 15 minutes. This solution was then centrifuged at 3000 x g for 15 minutes. Absorbance values of supernatants were recorded at 412 nm using a UV-VIS spectrophotometer. NPSH concentration was calculated using the published molar extinction coefficient of $13.1 \times 10^5\ M^{-1}cm^{-1}$.[11] Fifty mg of pooled lung tissue was analyzed for tissue protein concentration by the method of Lowry and co-workers.[12] Nonprotein sulfhydryls were standardized using lung tissue protein concentrations.

Comparisons of lung weight parameters or tissue biochemical measurements between exposed drug-treated mice and exposed PBS-treated mice were analyzed by a one-way ANOVA. If significant, the ANOVA was followed by a Newman-Keuls post hoc multiple comparison test. Survival was statistically evaluated using Chi square (χ^2) distribution. Calculations of odds ratios for survival were performed according to Kahn.[13] Data were considered statistically different at a significance level of $p \leq .05$.

Results

In this set of experiments all air exposed, mesna-treated mice survived for 24 hours. We did see an increase in survival rates in mice that received phosgene and were post-treated with mesna. Survival rates were significantly increased in the 834 mg/kg mesna + CG group over the CG + PBS group in each two-hour time segment starting at the four-hour time point and going through 8 hours, $p \leq .05$. In contrast, the 1,667 mg/kg mesna + CG group showed an increase in survival rate over the CG + PBS group only at the 8-hour time point, $p \leq .05$ (Figure 3).

Lung wet weight is an indication of the amount of edema produced in the lung. In our experiment, lung wet weight was significantly increased in phosgene-exposed mice compared with air-exposed mice (Figure 4); in some cases it was 2 to 3 times that of the air-exposed mice at the 12-hour time point. Even in mesna-treated mice at all concentrations, lung wet weight was not reduced enough to approach air-exposed mice. There does, however, appear to be a downward trend after the 334 mg/kg mesna dose in the lung wet weight. In fact, in this gravimetric assessment, the 1,667 mg/kg dose of mesna, when given to CG-exposed mice, significantly reduced the lung wet weight over that of the other doses of mesna.

The existence of pulmonary edema increases the proportion of packed red blood cells to plasma ratio and is a further indication of a severe lung fluid imbalance. Lungs taken from CG mice take on a hemorrhagic appearance and, because of the packed red blood cell hyperaggregation, may have increased dry weights. We dried both air-exposed and CG-exposed lungs for eight days and assessed the differences in their weights as an indication of lung damage (Figure 5). Lung dry weights for CG-exposed mice were significantly higher at all mesna concentrations than that of air-exposed mice at the 12-hour time point. In fact, they were almost double that of air-exposed mice regardless of the concentration of mesna. However, the lung dry weights of 1,667 mg/kg mesna + CG mice were significantly lower than that of the other mesna-treated mice or that of the PBS + CG mice. We saw the same phenomena in the wet weight to dry weight ratio (Figure 6). Wet/dry weight ratios for CG-exposed mice were three times that of air-exposed animals regardless of the mesna concentrations at the 12-hour time point. However, again at the 1,667 mg/kg mesna concentration, the wet/dry weight ratios were significantly reduced compared with other mesna-treated or PBS-treated CG-exposed mice.

Thiol status in the lung was determined by measuring lung tissue nonprotein-bound sulfhydryls. While there appears to be a small trend upward in the mesna + CG mice, the NPSH are not significantly increased over air-exposed mice for the three lower concentrations of mesna. However, in mice receiving 1,667 mg/kg of mesna + CG, there was a significant increase in NPSH over air-exposed mice (Figure 7).

Discussion

Phosgene is capable of widespread tissue destruction and cell death as well as reducing thiol containing glutathione levels in lung tissue.[14,15] In an attempt to increase survival rates of mice exposed to phosgene, we treated exposed mice with mesna (ip), a drug that has been shown to aid mucociliary clearance[1] and to have antioxidant properties.[4,5] We used large doses of mesna, 334, 834 and 1,667 mg/kg (the LD_{50} for mice is about 2,230 mg/kg, sc),[16] since we wanted to stay just below the LD_{50}. When we increased the dose of mesna to 3,334 mg/kg, 75% of the air-exposed mice died within 90 minutes (data not shown). At the concentrations of mesna that we used in this experiment (334, 834 and 1,667 mg/kg), mesna had little or no effect on air-exposed mice. Ormstad[3] found that after ip injections of mesna in rats, the plasma levels of free thiol groups were greatly reduced within the first hour after injection. For this reason, we injected the mice multiple times (five in all) to try to maintain constant levels of free thiols. Since the mice were getting the mesna in 0.5 ml injections, we wanted to space the injection times to allow the animals to absorb the liquid. Since we could not predict when the mice would die from the exposure to phosgene, some of the mice were injected just prior to death, whereas others died an hour or more after being injected.

Mesna, while increasing glutathione concentrations, may also increase intracellular levels of cysteine.[17] Lauterburg[17] saw only a transient rise of GSH as he combined mesna with ifosfamide, an alkylating chemotherapeutic drug. Mesna combines with ifosfamide metabolites detoxifying them, but rendering mesna unable to convert GSSG to GSH. Since we did not use mesna in combination with any other drugs we hoped to see a constant increase in GSH, and because mesna is also a mucolytic agent and has shown antioxidant properties,[4] we thought it to be a good candidate drug to protect against a phosgene challenge. We realized that the doses of mesna were very large and probably not realistic for use in the field; however, determination of survival rates was the main reason for this set of experiments.

Mesna did statistically increase survivability through 8 hours at the 834 mg/kg concentration, and although more mice given CG + mesna survived after 8 hours than did mice given CG + PBS, there was no statistical difference between the two groups. As mentioned above, Lauterburg also saw an increase in cysteine levels. This rise in cysteine may have led to cysteine toxicity and as a result caused increased mortality. In fact, Olney saw that giving L-cysteine sc, 1, 2 and 3 mg/g to 8- to 10-day-old mice can be lethal.[18] It is also known that cysteine may be spontaneously oxidized to cysteine disulfide and H_20_2, causing damage to cell membranes.[19] We may have been at a critical level of mesna where the 834 mg/kg dose was beneficial to mice and the 1,667 mg/kg dose was enough to cause too great a rise in cysteine, too much for the liver to detoxify. It could be possible that the increase in cysteine caused by mesna may have been too much for an already compromised animal to endure.

Phosgene has been shown to increase TNF-production from macrophages,[20] compromise the immune system and cause lung infection,[21] increase plasma iron,[22] and increase arachidonic acid mediators;[23] therefore, many obstacles would have to be overcome to increase survival due to phosgene exposure. Sciuto *et al.*[24] found that an intratracheal administration of N-acetylcysteine after exposure to phosgene elevated GSH levels in rabbits. Sciuto also found that N-acetylcysteine prevented edema formation,

peptide leukotrine production and lipid peroxidation in rabbits. Our group administered N-acetylcysteine interperitoneally (ip) to mice after exposure to phosgene and saw little GSH elevation (data not shown). Could this phenomenon be due to the route of administration, intratracheal vs ip? It would appear that delivering a drug directly to the site of injury would be beneficial as opposed to administering the drug to another site. Could our lack of GSH elevation be due to our use of a different species? In much the same vein, would mesna benefit from a different route of administration? It would appear that raising GSH levels is not enough to ensure survival.

In conclusion, although mesna conveys increased survivability through 8 hours, there is insufficient evidence of overall improved outcome at these concentrations with its use in the treatment of CG exposure using this paradigm.

Figure 1. Structure of Mesna

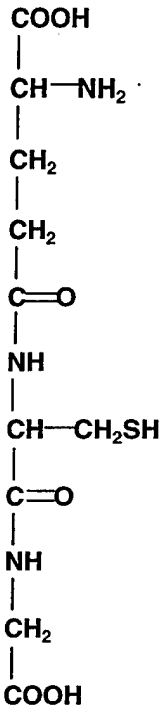

Figure 2. Structure of GSH

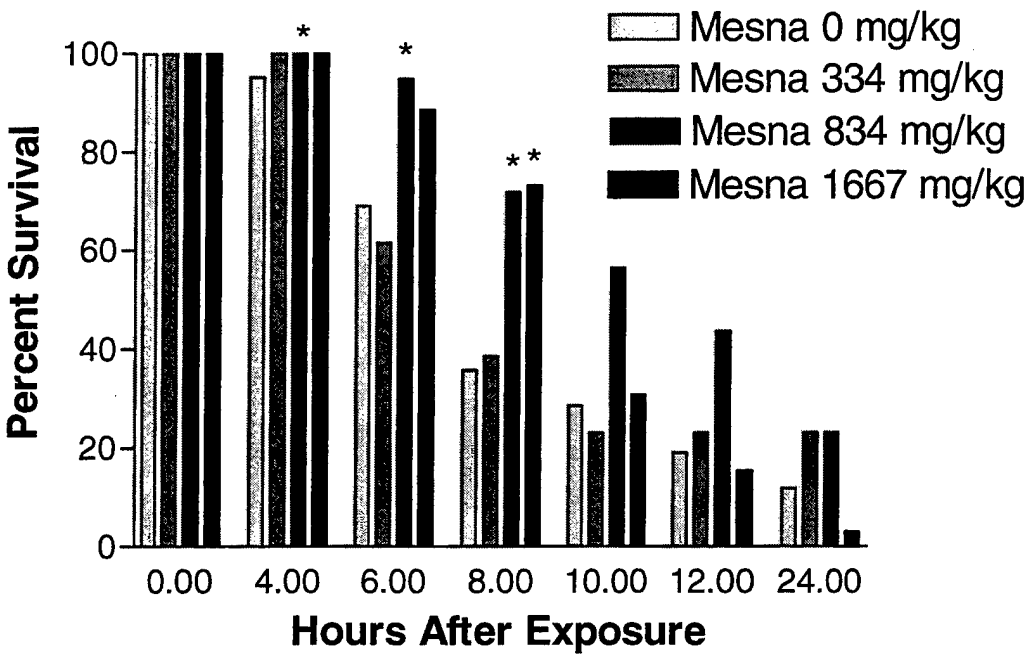

Figure 3. Survival rates for varying concentrations of mesna.

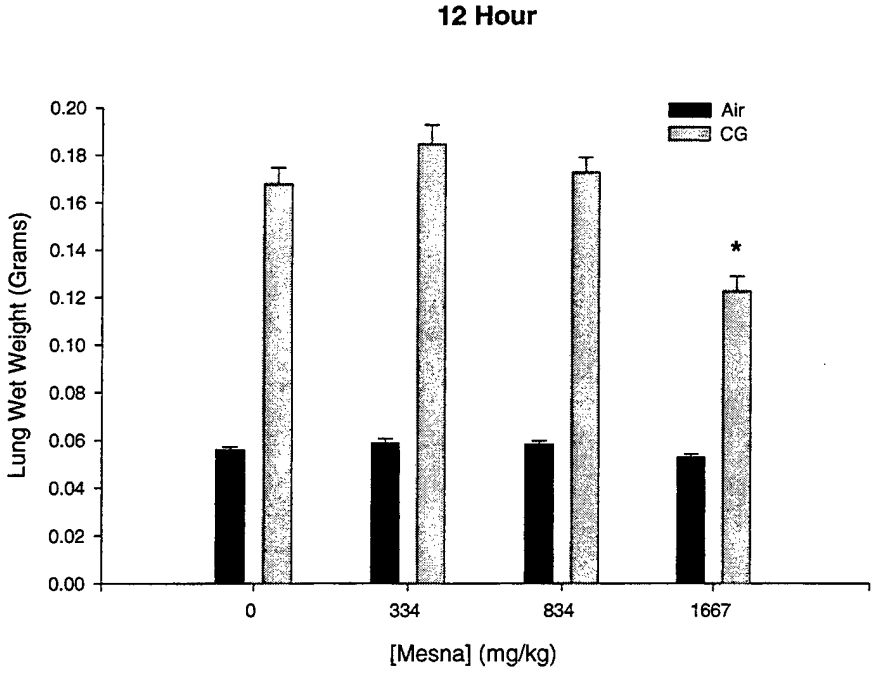

Figure 4. Lung wet weight at 12 hours.

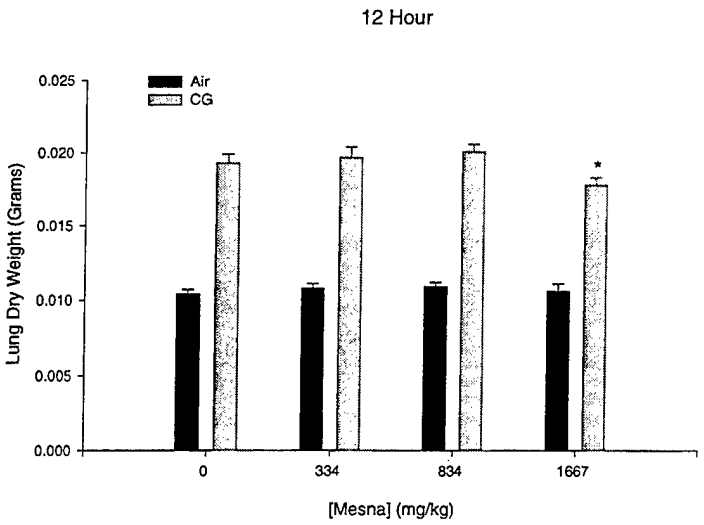

Figure 5. Dry weights for lungs exposed to phosgene and air at 12 hours.

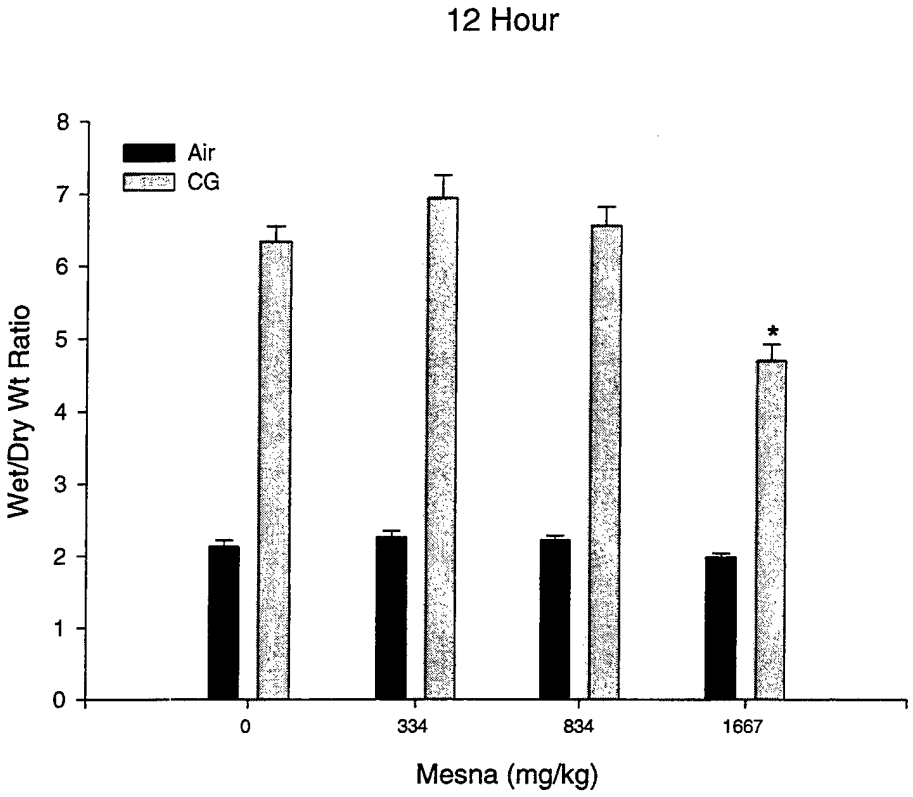

Figure 6. Lung wet weight divided by dry weight.

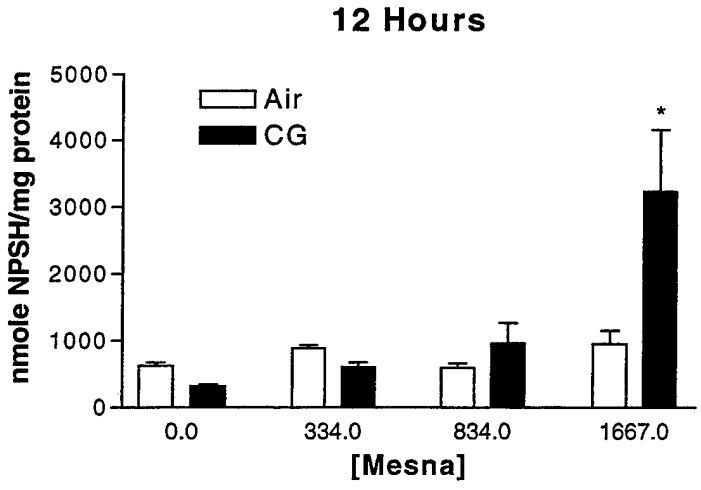

Figure 7. Non-protein sulfhydryls corrected for protein.

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